

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 May 2000 (24.05.00)	
International application No. PCT/GB99/03258	Applicant's or agent's file reference 100280/JND/CH
International filing date (day/month/year) 01 October 1999 (01.10.99)	Priority date (day/month/year) 01 October 1998 (01.10.98)
Applicant SCHMIDT, Günter et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

27 April 2000 (27.04.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Juan Cruz Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PRELIMINARY EXAMINING AUTHORITY

S, Jeffrey N.
WHITE & FARRER
Joughtry Street
JNDON WC1N 2LS
GRANDE BRETAGNE

RECEIVED

1 FEB 2001

An'sd.....

PCT
DE 20010201

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing (day/month/year)	29.01.2001
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Applicant's or agent's file reference 100280JND/CH	IMPORTANT NOTIFICATION
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International application No. PCT/GB99/03258	International filing date (day/month/year) 01/10/1999	Priority date (day/month/year) 01/10/1998
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Applicant BRAX GROUP LIMITED et al.
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1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
---------------------------------------	--------------------

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Pedersen, C

Tel.+49 89 2399-8063 8161



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 100280JND/CH	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/03258	International filing date (day/month/year) 01/10/1999	Priority date (day/month/year) 01/10/1998	
International Patent Classification (IPC) or national classification and IPC G01N33/68			
Applicant BRAZ GROUP LIMITED et al.			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 1 sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 			
Date of submission of the demand 27/04/2000	Date of completion of this report 29.01.2001		
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Jacques, P Telephone No. +49 89 2399 8934 		

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03258

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*):

Description, pages:

1-24 as originally filed

Claims, No.:

5-17 as originally filed

1-4 with telefax of 22/01/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03258

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims 1-17
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-17
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-17
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 98 32876 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 30 July 1998 (1998-07-30)

2. The documents "Identification of the active site serine of penicillin-binding...., Sun, Yiping et al., J. Mass. Spectrom..., Vol. 33 (10), pp 1009-1016, 1998" and " An algorithm for the identification of proteins..., Korostensky, Chantal et al., Vol. 19(11), pp 1933-1940 (1998)" cited as P-documents in the International Search Report are not to be regarded as state of the art according to Article 33(2) EPC, as the date of priority claimed can be allowed for the relevant parts of the present application.
3. Documents "BENNETT, GUDRUN S. ET AL: 'Identification of Ser-Pro and Thr-Pro phosphorylation sites in chicken neurofilament-M tail domain' J. NEUROCHEM. (1997), 68(2), 534-543", "PAPAC, DAMON I. ET AL: 'Palmitylation of a G- protein coupled receptor. Direct analysis by tandem mass spectrometry' J. BIOL. CHEM. (1992), 267(24), 16889-94, and "FURUYA, MAMI ET AL: 'The primary structure of human EGF produced by genetic engineering, studied by high-performance tandem mass spectrometry.' BIOCHEM. BIOPHYS. RES. COMMUN. (1989), 163(2), 1100-6", all cited as X-documents in the International Search Report, have not been considered as pertinent in the art as all of them fail to disclose the isolation of one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the polypeptide from which it was fragmented and the repetition of steps (a)-(c) with a second cleavage agent.
4. As amended claims 1-4 filed on 22.01.2001 do not contain subject-matter which extends beyond the content of the application as originally filed, they can be considered to meet the requirements of Articles 19(2) and 34(2)(b).
5. As the particular combination of features of independent claim 1 is not disclosed in

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

6. Moreover, the subject-matter of claim 1 appears to involve an inventive step in the sense of Article 33(3) for the following reasons:

the closest state of the art is considered to result from document D1.

This document discloses a method for characterising polypeptides which comprises (a) treating a sample comprising a population of one or more polypeptides with a cleavage agent, (b) isolating a population of the peptide fragments which bear at one end a reference terminus comprising either only an C-terminus or only an N-terminus and (c) determining a signature sequence of at least some of the isolated fragments (see abstract), the said signature being the determination of peptide mass fingerprints for the population of signature peptides generated, the said determination being made by mass spectrometry (see page 11, lines 8-16), thus characterising one or more polypeptides in the sample.

The subject-matter of claim 1 is distinguished therefrom by the following feature: steps (a)-(c) are repeated using a second cleavage agent that cleaves the starting material at a different site from the first cleavage agent.

The technical effect of this distinguishing feature result in producing N- or C-terminus fragments of different masses.

The technical problem to be solved by the invention was therefore to resolve mass ambiguities in a number of terminal peptide peaks from a number of different initial proteins.

The problem posed has convincingly been solved by repeating steps (a)-(c) of the method of claim 1 using a second cleavage agent that cleaves a different site from the first cleavage agent.

Although document D2 suggests that to resolve mass ambiguities, a digestion of further starting material using a different digestion technique to produce cleavage at different site can be performed, the skilled person would not have been motivated to consult D2 which is concerned with extracting sequence information from ragged ends and not concerned with distinguishing a mixture of polypeptides.

Thus, as no specific instructions or indications to repeat and modify the cleaving steps to distinguish a population of polypeptides can be found in the cited prior art,

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

the subject-matter of claim 1 can be considered to involve an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 2-4 and 8-15.

7. As the particular combination of features of independent claim 5 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

8. Moreover, the subject-matter of the said claim appears to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

the subject-matter of the claim 5 is distinguished from the prior art D1 (see above point 5) in that a capping step is used, the said step being repeated with a different capping agent.

The technical effect of this distinguishing features result in producing terminal fragments of different masses.

The technical problem to be solved by the invention was therefore to resolve mass ambiguities in a number of terminal peptide peaks from a number of different initial proteins.

The problem posed has convincingly been solved by introducing a plurality of capping groups, the said capping groups having different masses.

As no indications suggesting using a plurality of capping groups to solve the above mentioned problem can be found in the prior art, the subject-matter of claim 5 can be considered to involve an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 6 and 7.

9. As the particular combination of features of independent claims 16 and 17 is not disclosed in any cited prior art, the subject-matter of the said claims would appear to be novel (Article 33(2) PCT).

10. Moreover, as the subject-matter of the said claims relates to the application of the method disclosed in claim 1, for determining the expression of a protein in a tissue (claim 16) or assaying for one or more specific polypeptide in a sample (claim 17),

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

the same reasoning as for the said claim applies to claims 16 and 17 which are new (Article 33(2) PCT) and involve an inventive step (Article 33(3) PCT).

Re Item VIII

Certain observations on the international application

1. The vague and imprecise statement in the description on page 12, line 11 ("the scope of this invention") implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity of the claims (Article 6 PCT) when used to interpret them (see Guidelines, C-III, 4.3a).

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DANIELS, Jeffrey N.
PAGE WHITE & FARRER
54 Doughty Street
LONDON WC1N 2LS
GRANDE BRETAGNE

RECEIVED

12 JUN 2000

Ans'd.....

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing (day/month/year)	08.06.2000
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Applicant's or agent's file reference
100280JND/CH

REPLY DUE

within 3 month(s)
from the above date of mailing

International application No.
PCT/GB99/03258

International filing date (day/month/year)
01/10/1999

Priority date (day/month/year)
01/10/1998

International Patent Classification (IPC) or both national classification and IPC

G01N33/68

Applicant

BRAX GROUP LIMITED et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I Basis of the opinion
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain document cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 01/02/2001.

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner

Jacques. P

Formalities officer (incl. extension of time limits)
Borinski, W
Telephone No. +49 89 2399 8237



I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-24 as originally filed

Claims, No.:

1-17 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims	1-17 (Yes)
Inventive step (IS)	Claims	1-3, 8-17 (No); 4-7 (Yes)
Industrial applicability (IA)	Claims	1-17 (Yes)

2. Citations and explanations

see separate sheet

WRITTEN OPINION

International application No. PCT/GB99/03258

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 98 32876 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 30 July 1998 (1998-07-30) cited in the application
D2: GB-A-2 168 478 (SCAN LIMITED M) 18 June 1986 (1986-06-18)

2. The documents "Identification of the active site serine of penicillin-binding...., Sun, Yiping et al., J. Mass. Spectrom..., Vol. 33 (10), pp 1009-1016, 1998" and " An algorithm for the identification of proteins..., Korostensky, Chantal et al., Vol. 19(11), pp 1933-1940 (1998)" cited as P-documents in the International Search Report are not to be regarded as state of the art according to Article 33(2) EPC, as the date of priority claimed can be allowed for the relevant parts of the present application.
3. Documents "BENNETT, GUDRUN S. ET AL: 'Identification of Ser-Pro and Thr-Pro phosphorylation sites in chicken neurofilament-M tail domain' J. NEUROCHEM. (1997), 68(2), 534-543", "PAPAC, DAMON I. ET AL: 'Palmitylation of a G- protein coupled receptor. Direct analysis by tandem mass spectrometry' J. BIOL. CHEM. (1992), 267(24), 16889-94, and "FURUYA, MAMI ET AL: 'The primary structure of human EGF produced by genetic engineering, studied by high-performance tandem mass spectrometry.' BIOCHEM. BIOPHYS. RES. COMMUN. (1989), 163(2), 1100-6", all cited as X-documents in the International Search Report, have not been considered as pertinent in the art as all of them fail to disclose the isolation of one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the polypeptide from which it was fragmented and the repetition of steps (a)-(c) with a second cleavage agent.
4. As the particular combination of features of independent claim 1 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).
5. However, the subject-matter of independent claim 1 does not involve an inventive

step in the sense of Article 56 EPC for the following reasons:

the closest state of the art is considered to result from document D1.

This document discloses a method for characterising polypeptides which comprises (a) treating a sample comprising a population of one or more polypeptides with a cleavage agent, (b) isolating a population of the peptide fragments which bear at one end a reference terminus comprising either only an C-terminus or only an N-terminus and (c) determining a signature sequence of at least some of the isolated fragments (see abstract), the said signature being the determination of peptide mass fingerprints for the population of signature peptides generated, the said determination being made by mass spectrometry (see page 11, lines 8-16), thus characterising one or more polypeptides in the sample.

The subject-matter of claim 1 is distinguished therefrom by the following feature: steps (a)-(c) are repeated using a second cleavage agent that cleaves at a different site from the first cleavage agent.

The technical effect of this distinguishing feature result in producing terminal fragments of different masses.

The technical problem to be solved by the invention was therefore to enhance the resolution of the method.

The person skilled in the art would turn to document D2 for the solution of this particular problem. This document discloses the determination of the amino acid content of a peptide fragment based on the mass of the said peptide fragment (page 1, right column, lines 110-121).

This document is concerned with a similar problem that is that sometimes the mass of a fragment may convey ambiguous information (page 2, left column, lines 1-2). It is suggested that to solve this problem, a digestion of further starting material using a different digestion technique to produce cleavage at different sites (thus producing fragments of different masses) can resolve the ambiguity (see page 2, left column, lines 1-9), thus enhancing the resolution of the method.

This suggestion essentially corresponds to the feature which distinguishes the invention from the state of the art.

Thus, the skilled person would have applied the solution disclosed in D2 to solve the above mentioned problem without the exercise of any inventive skill.

Therefore, the subject-matter of claim 1 does not meet the requirements of Article

33(3) PCT.

6. Dependent claims 2, 3, 8-15 do not appear to contain any additional features which, in combination with claim 1, meet the requirements of inventive steps as all the features of these claims are either conventional in the art (claims 2, 8, 15) or disclosed in the prior art (see D1, page 13, "C-terminal sequencing paragraph" and figure 1 for claim 3, page 7, lines 2-9 for claims 11-12; page 5, lines 1-3 for claims 13-14; see reference cited in the description, page 3, second paragraph, for claims 9-11).

The same applies to claims 16 and 17 as it falls within the normal design capabilities of the skilled man to apply the method of claim 1, directed to the characterisation of a polypeptide or a population of polypeptides, for determining the expression of a protein in a tissue or assaying for one or more specific polypeptide in a sample.

7. The subject-matter of claim 4 would appear to involve an inventive step in the sense of Article 33(3) PCT.

The subject-matter of the said claim is distinguished from the prior art by the following feature: the capping step and steps (a)-(c) are repeated one, two or more times, each time introducing capping groups at the same side chains as the previous capping steps, but using capping groups having different mass than the corresponding capping groups used in the previous capping steps.

The technical effect of this distinguishing feature result in producing terminal fragments of different masses.

The technical problem to be solved by the invention was therefore to enhance the resolution of the method.

The problem posed has convincingly been solved by using a plurality of capping groups, each having a different mass.

As no indications suggesting using a plurality of capping group to solve the above mentioned problem can be found in the prior art, the subject-matter of claim 4 can be considered to involve an inventive step in the sense of Article 33(3) PCT.

8. As the method of independent claim 5 appears to be based on the same principle as the method of claim 4 with the exception of step (d) (the use of a second cleavage agent that cleaves at a different site), the same reasoning as for claim 4 applies to

claim 5 (see point 7).

The said claim is therefore considered to be new (Article 33(2) PCT) and to involve an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 6, 7 (see however the objection raised under Article 6 PCT under Item VIII).

Re Item VII

Certain defects in the international application

1. To meet the requirements of Rule 5.1(a)(ii) PCT, the document D2 should be identified in the description and the relevant background art disclosed therein should be briefly discussed.

Re Item VIII

Certain observations on the international application

1. Should the applicant overcome the aforesaid objections under article 33(3) PCT by restricting the scope of the first independent claim to the features of claim 4, the following objection would apply: the subject-matter of new independent claim 1 and claim 7 would appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect to the terminology used for the features of that subject-matter. The aforesaid claims therefore would lack conciseness. Moreover, lack of clarity of the claims as a whole would arise, since the plurality of independent claims would make it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection.

Hence, new independent claim 1 and claim 7 would not meet the requirements of Article 6 PCT.

2. The vague and imprecise statement in the description on page 12, line 11 ("the scope of this invention") implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity of the claims (Article 6 PCT) when used to interpret them (see Guidelines, C-III, 4.3a). This

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/GB99/03258

statement should therefore be amended to remove this inconsistency.



EPA/EPO/OEB
D-80298 München
+49 89 2399-0
TX 523 656 epmu d
FAX +49 89 2399-4465

Europäisches
Patentamt

Generaldirektion 2

Europ. Patent Office

Directorate General 2

Office européen
des brevets

Direction Générale 2

Correspondence with the EPO on PCT Chapter II demands

In order to ensure that your PCT Chapter II demand is dealt with as promptly as possible you are requested to use the enclosed self-adhesive labels with any correspondence relating to the demand sent to the Munich Office.

One of these labels should be affixed to a prominent place in the upper part of the letter or form etc. which you are filing.

Our ref: 100280/CMH/k1 9 October 2000

*European Patent Attorneys
Chartered Patent Attorneys
Trade Mark Agents*

Your ref:

BY FAX & POST

European Patent Office
D-80298 Munich
Germany

*Page White & Farmer
54 Doughty Street
London WC1N 2LS
Telephone 020 7831 7929
Facsimile 020 7831 8040
email@pagewhite.co.uk*

Dear Sirs,

**Re: International Patent Application No. PCT/GB99/03258
Claiming priority from GB Appln. 9821393.7
Protein Profiling III**

In response to the Written Opinion dated 8 June 2000, and further to our letter requesting extension of the term for response of 7 September 2000, the following comments are provided.

The Examiner has accepted the novelty of the claims of the present application. However, the Examiner has argued that some of the claims, including claim 1, lack an inventive step in view of D1 taken in combination with D2.

Specifically, the Examiner has argued that the subject matter of claim 1 is distinguished from D1 in that steps (a) – (c) are repeated using a second cleavage agent. This allows enhancement of the resolution of the method. The Examiner points out that D2 suggests using a different digestion technique to produce smaller fragments to resolve ambiguities in mass information. The Examiner concludes that to increase the resolution of the method of D1, the skilled person could use further different cleavage agents as mentioned in D2 to arrive at the present method.

Directors
R Palmer
D J Richards
P D Jenkins
Mrs V R Driver
J N Daniels
Miss K C Style
Ms N Shackleton
P R Slingsby

However, it is respectfully submitted that the above interpretation of D2 is not entirely correct, such that a combination of D1 and D2 does not lead to the invention as presently claimed. Specifically, D2 is concerned with identifying ragged ends in a known protein. Ragged ends are defined as being terminal fragments which are missing several peptides, or have several additional peptides than the normal protein. D2 achieves this by examining the terminal fragments of a known protein by mass spectrometry. D2 teaches that the use of different cleavage agents is intended for determining the *sequence* of the ragged end peptides. This is because the composition of the peptide can almost always be determined by the mass alone (see page 1, column 2,

Associate Directors
W J Neobard
J P Cornish
C M Hill
J P Ruuskanen

*D Williams
J C Boakes*

Consultant
A Pendlebury

penultimate paragraph). The ambiguity of mass information is hardly a problem in D1, since a population of polypeptides is not being investigated, but rather single polypeptides are being investigated to determine whether they have ragged ends or not. Clearly where there is a single polypeptide it is much less likely that there will be enough different fragments to give rise to mass ambiguities. The mass ambiguities referred to on page 2, column 1, paragraph 1 of D2 are only mentioned for the case where the mass of the peptide does not uniquely determine the *sequence* of the peptide. It is important to remember that it is the sequence of the peptide which is of primary importance in D2.

In contrast, the present invention envisages resolving a large number of polypeptides by examining an even greater number of polypeptide fragments containing the termini of these polypeptides. If the terminal peptide products of one cleavage agent do not all have a unique mass, then unlike D2 it is not the sequence of the protein from which the peptide is derived which is ambiguous, but rather the complete identity of the protein remains ambiguous. D2 does not disclose that the use of further cleavage agents will be able to resolve one different polypeptide from the next, but rather envisages resolving particular sequence information within a single polypeptide agent which has a ragged end, but in which the sequence of the ragged end is ambiguous. The problem with regard to D2 is greatly simplified since it only considering a single polypeptide at a time.

Bearing the above in mind, the approach of D2 would not address the problem solved by the present invention which is to resolve mass ambiguities in a number of terminal peptide peaks from a number of different initial proteins. Thus, a combination of the teaching of D1 and D2 would not lead to the present invention. Moreover, the skilled person would not have consulted D2, which is concerned with extracting sequence information form ragged ends and not concerned with distinguishing a mixture of proteins.

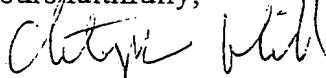
Having regard to all of the above, it is respectfully submitted that the present claims are associated with an inventive step.

It is believed that the above comments clearly answer all of the substantive objections raised in the Official Action.

Accordingly, favourable reconsideration of the application is petitioned.

Please acknowledge receipt of this letter by returning the top copy of the enclosed Form 1037.

Yours faithfully,



Dr. Christopher M. Hill
(Authorised Representative)

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 100280JND/CH	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/03258	International filing date (day/month/year) 01/10/1999	Priority date (day/month/year) 01/10/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant BRAX GROUP LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 27/04/2000	Date of completion of this report 29.01.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Jacques, P Telephone No. +49 89 2399 8934



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03258

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17.)*):

Description, pages:

1-24 as originally filed

Claims, No.:

5-17 as originally filed

1-4 with telefax of 22/01/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03258

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 98 32876 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 30 July 1998 (1998-07-30)

2. The documents "Identification of the active site serine of penicillin-binding...., Sun, Yiping et al., J. Mass. Spectrom..., Vol. 33 (10), pp 1009-1016, 1998" and " An algorithm for the identification of proteins..., Korostensky, Chantal et al., Vol. 19(11), pp 1933-1940 (1998)" cited as P-documents in the International Search Report are not to be regarded as state of the art according to Article 33(2) EPC, as the date of priority claimed can be allowed for the relevant parts of the present application.
3. Documents "BENNETT, GUDRUN S. ET AL: 'Identification of Ser-Pro and Thr-Pro phosphorylation sites in chicken neurofilament-M tail domain' J. NEUROCHEM. (1997), 68(2), 534-543", "PAPAC, DAMON I. ET AL: 'Palmitylation of a G- protein coupled receptor. Direct analysis by tandem mass spectrometry' J. BIOL. CHEM. (1992), 267(24), 16889-94, and "FURUYA, MAMI ET AL: 'The primary structure of human EGF produced by genetic engineering, studied by high-performance tandem mass spectrometry.' BIOCHEM. BIOPHYS. RES. COMMUN. (1989), 163(2), 1100-6", all cited as X-documents in the International Search Report, have not been considered as pertinent in the art as all of them fail to disclose the isolation of one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the polypeptide from which it was fragmented and the repetition of steps (a)-(c) with a second cleavage agent.
4. As amended claims 1-4 filed on 22.01.2001 do not contain subject-matter which extends beyond the content of the application as originally filed, they can be considered to meet the requirements of Articles 19(2) and 34(2)(b).
5. As the particular combination of features of independent claim 1 is not disclosed in

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

6. Moreover, the subject-matter of claim 1 appears to involve an inventive step in the sense of Article 33(3) for the following reasons:

the closest state of the art is considered to result from document D1.

This document discloses a method for characterising polypeptides which comprises (a) treating a sample comprising a population of one or more polypeptides with a cleavage agent , (b) isolating a population of the peptide fragments which bear at one end a reference terminus comprising either only an C-terminus or only an N-terminus and (c) determining a signature sequence of at least some of the isolated fragments (see abstract), the said signature being the determination of peptide mass fingerprints for the population of signature peptides generated, the said determination being made by mass spectrometry (see page 11, lines 8-16), thus characterising one or more polypeptides in the sample.

The subject-matter of claim 1 is distinguished therefrom by the following feature: steps (a)-(c) are repeated using a second cleavage agent that cleaves the starting material at a different site from the first cleavage agent.

The technical effect of this distinguishing feature result in producing N- or C-terminus fragments of different masses.

The technical problem to be solved by the invention was therefore to resolve mass ambiguities in a number of terminal peptide peaks from a number of different initial proteins.

The problem posed has convincingly been solved by repeating steps (a)-(c) of the method of claim 1 using a second cleavage agent that cleaves a different site from the first cleavage agent.

Although document D2 suggests that to resolve mass ambiguities, a digestion of further starting material using a different digestion technique to produce cleavage at different site can be performed, the skilled person would not have been motivated to consult D2 which is concerned with extracting sequence information from ragged ends and not concerned with distinguishing a mixture of polypeptides.

Thus, as no specific instructions or indications to repeat and modify the cleaving steps to distinguish a population of polypeptides can be found in the cited prior art,

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

the subject-matter of claim 1 can be considered to involve an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 2-4 and 8-15.

7. As the particular combination of features of independent claim 5 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

8. Moreover, the subject-matter of the said claim appears to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

the subject-matter of the claim 5 is distinguished from the prior art D1 (see above point 5) in that a capping step is used, the said step being repeated with a different capping agent.

The technical effect of this distinguishing features result in producing terminal fragments of different masses.

The technical problem to be solved by the invention was therefore to resolve mass ambiguities in a number of terminal peptide peaks from a number of different initial proteins.

The problem posed has convincingly been solved by introducing a plurality of capping groups, the said capping groups having different masses.

As no indications suggesting using a plurality of capping groups to solve the above mentioned problem can be found in the prior art, the subject-matter of claim 5 can be considered to involve an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 6 and 7.

9. As the particular combination of features of independent claims 16 and 17 is not disclosed in any cited prior art, the subject-matter of the said claims would appear to be novel (Article 33(2) PCT).

10. Moreover, as the subject-matter of the said claims relates to the application of the method disclosed in claim 1, for determining the expression of a protein in a tissue (claim 16) or assaying for one or more specific polypeptide in a sample (claim 17),

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03258

the same reasoning as for the said claim applies to claims 16 and 17 which are new (Article 33(2) PCT) and involve an inventive step (Article 33(3) PCT).

Re Item VIII

Certain observations on the international application

1. The vague and imprecise statement in the description on page 12, line 11 ("the scope of this invention") implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity of the claims (Article 6 PCT) when used to interpret them (see Guidelines, C-III, 4.3a).

CLAIMS:

1. A method for characterising a population of polypeptides, which method comprises:
 - (a) contacting a sample comprising polypeptides with a first cleavage agent to generate polypeptide fragments;
 - (b) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the polypeptide from which it was fragmented;
 - (c) identifying the isolated fragments by mass spectrometry;
 - (d) repeating steps (a)-(c) on the sample using a second cleavage agent that cleaves at a different site from the first cleavage agent; and
 - (e) characterising the polypeptides in the sample from the fragments identified in steps (c) and (d).
2. A method according to claim 1, wherein the step (d) comprises repeating steps (a)-(c) two or more times, each time using a further cleavage agent that cleaves at a different site from the previous cleavage agents.
3. A method according to claim 1 or claim 2, comprising a further capping step prior to step (a), which capping step comprises reacting the polypeptides in the sample with one or more capping agents to introduce capping groups on one or more reactive side chains of the polypeptides.
4. A method according to claim 3, wherein the capping step and steps (a)-(c) are repeated one, two, or more times, each time introducing capping groups at the same side chains as the previous capping steps, but using capping groups having different mass than the corresponding capping groups used in the previous capping steps.

AMENDED SHEET

PATENT COOPERATION TREATY

PCT

W/V

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 100280JND/CH	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, Item 5 below.	
International application No. PCT/GB 99/ 03258	International filing date (day/month/year) 01/10/1999	(Earliest) Priority Date (day/month/year) 01/10/1998
Applicant BRAX GROUP LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the International search was carried out on the basis of the International application in the language in which it was filed, unless otherwise indicated under this item.
 - the International search was carried out on the basis of a translation of the International application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any nucleotide and/or amino acid sequence disclosed in the International application, the International search was carried out on the basis of the sequence listing :
 - contained in the International application in written form.
 - filed together with the International application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the International application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of invention is lacking (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

CHARACTERISING POLYPEPTIDES THROUGH CLEAVAGE AND MASS SPECTROMETRY

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

GB 99/03258

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 168 478 A (SCAN LIMITED M) 18 June 1986 (1986-06-18)	1,2,9, 12-14, 16,17
Y	column 2, line 15 - line 39 ---	1,3,8-11
Y	WO 98 32876 A (BRAX GENOMICS LTD ; THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 30 July 1998 (1998-07-30) cited in the application claims 1-11 ---	1,3,8-11

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

15 February 2000

Date of mailing of the International search report

28/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5618 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Hart-Davis, J

INTERNATIONAL SEARCH REPORT

International Application No

GB 99/03258

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BENNETT, GUDRUN S. ET AL: "Identification of Ser-Pro and Thr-Pro phosphorylation sites in chicken neurofilament-M tail domain" J. NEUROCHEM. (1997), 68(2), 534-543 , XP000874042 abstract figure 3 table 1 ---	1,2, 12-14
X	PAPAC, DAMON I. ET AL: "Palmitylation of a G- protein coupled receptor. Direct analysis by tandem mass spectrometry" J. BIOL. CHEM. (1992), 267(24), 16889-94 , XP000867521 the whole document ---	1,2, 12-14
X	FURUYA, MAMI ET AL: "The primary structure of human EGF produced by genetic engineering, studied by high-performance tandem mass spectrometry." BIOCHEM. BIOPHYS. RES. COMMUN. (1989), 163(2), 1100-6 , XP000867522 the whole document ---	1,2, 12-14
P,X	SUN, YIPING ET AL: "Identification of the active site serine of penicillin-binding 2a from methicillin-resistant Staphylococcus aureus by electrospray mass spectrometry" J. MASS SPECTROM. (1998), 33(10), 1009-1016 , XP000874145 the whole document ---	1,2, 12-14
P,X	KOROSTENSKY, CHANTAL ET AL: "An algorithm for the identification of proteins using peptides with ragged N- or C-termini generated by sequential endo- and exopeptidase digestions" ELECTROPHORESIS (1998), 19(11), 1933-1940 , XP000878845 the whole document ---	1,2, 12-14
A	WO 95 25281 A (UNIV WASHINGTON ;YATES JOHN R III (US); ENG JAMES K (US); LINK AND) 21 September 1995 (1995-09-21) examples 1-3 ---	1
A	WO 96 36986 A (PERSEPTIVE BIOSYSTEMS INC) 21 November 1996 (1996-11-21) claims 1,9-14,20,21 -----	1,13,14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

GB 99/03258

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB 2168478	A 18-06-1986	US 4701419	A	20-10-1987
WO 9832876	A 30-07-1998	AU 5674598	A	18-08-1998
WO 9525281	A 21-09-1995	US 5538897	A	23-07-1996
		CA 2185574	A	21-09-1995
		EP 0750747	A	02-01-1997
		JP 9510780	T	28-10-1997
		US 6017693	A	25-01-2000
WO 9636986	A 21-11-1996	US 5869240	A	09-02-1999
		EP 0827628	A	11-03-1998
		US 5827659	A	27-10-1998
		US 5821063	A	13-10-1998